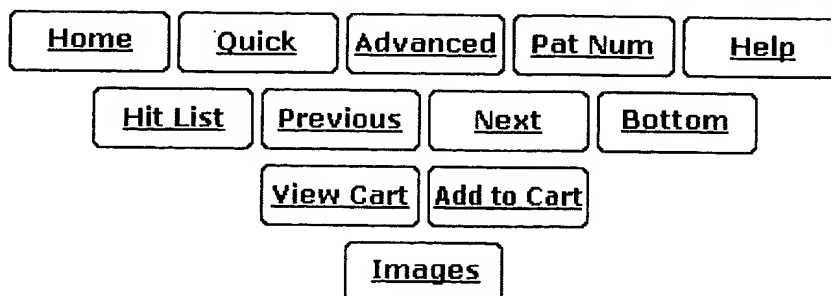


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United States Patent
Haugland , et al.**5,362,628**
November 8, 1994

Fluorescent haloalkyl derivatives of reporter molecules well retained in cells**Abstract**

The subject invention provides a method for analyzing the metabolic activity in cells by improving the retention of a detectable reporter molecule only in intact cells where a particular enzyme is present. In particular, improved retention results from a two part process involving conjugation of haloalkyl-substituted derivatives of a *reporter molecule* with intracellular cysteine-containing peptides while unblocking the *reporter molecule*. The method for analyzing metabolic activity of cells involves the use of a substrate having the form XR-REPORTER-BLOCK wherein -BLOCK is a group selected to be removable by action of a specific analyte, to give REPORTER spectral properties different from those of the substrate, *-REPORTER- is a molecule* that, when no longer bound to BLOCK by a BLOCK-REPORTER bond, has spectral properties different from those of the substrate, and XR-- is a haloalkyl moiety that can covalently react with an intracellular thiol (Z--S--H) to form a thioether conjugate (Z--S--R--). After the substrate enters the cells, the analyte removes BLOCK to make REPORTER detectable by the change in spectral properties, and the haloalkyl XR reacts with the intracellular thiol to form the thioether conjugate inside the cells, which is well-retained in the cells.

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